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Sciences of Phytochemistry

Pharmacognostic Study and Hepatoprotective Activity of the Methanolic Extract and Fractions of Leaves of *Picralima nitida* Apocyanaceae

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Keywords: Picralima nitida, Pharmacognostic, Hepatoprotective, Hepatotoxicity .

Abstract: *Picralima nitida* the only species in the genus Picralima belongs to the *Apocyanaceae* family. It is widely known for its medicinal purposes. The aim of the study was to investigate pharmacognostic parameters of the leaf and evaluate the hepatoprotective activity against carbon tetrachloride induced hepatotoxicity using Swiss Albino mice. The physicochemical evaluation indicated 11.75% moisture content, 8.50% total ash, 9.50% acid insoluble ash, 4.00% water soluble ash, 13.75% alcohol extractive value and 11.00% water extractive value. Macroscopic analysis on the fresh leaves revealed an odourless green plant with bitter taste. Microscopic examination indicated the presence of calcium oxalate crystals, starch grains, epidermal cells, xylem, parenchyma cells, paracytic stomata and palisade tissue. Chemomicroscopic evaluation indicated the presence of oxalate crystals, starch grains, lignified tissues, tannins, cellulose, protein and oil. The acute toxicity result revealed that *P. nitida* had no adverse effect in Swiss Albino mice. The ethyl acetate fraction had hepatoprotective ability on liver enzymes (alanine transaminase, aspartate aminotransferase, alkaline phosphatase) and can produce the same result as ascorbic acid (standard).

Introduction

Liver is the largest organ, accounting for approximately 2% to 3% of average body weight (1). It functions as a centre for metabolism of nutrients, excretion of waste metabolites and controls the flow and safety of substances absorbed from the digestive system before distribution to the systemic circulatory system (2, 3). According to the World Health Organization, an estimated 354 million people were reported to be living with hepatitis infection and for most, testing and treatment remain beyond reach (4). The symptoms of liver disease may include jaundice, abdominal pain and swelling, swelling in the legs and ankles, itchy skin, dark urine colour among other signs.

Researches on plants have shown that plants harbour in them bioactive phytochemicals (5-7) and from plants have been isolated phytocompounds (7). *Picralima nitida* (Stapf, see **Figure 1**) also known as the Akuamma plant is found in tropical African countries such as Ivory Coast, Nigeria, Uganda, and Gabon (8). It is popularly known as Abeere in the Southwestern part of Nigeria among the Yoruba people (9-11). The plant is known for its medicinal purposes and is used in traditional medicine for the treatment and management of diseases such as malaria, abscesses, hepatitis, pneumonia, diabetes, and hypertension (10-12). The previous works done on the leaves, stem bark, fruits, seeds and pods of *P. nitida* revealed polyphenols, peptide, amide, ester, terpenoids, and indole, alkaloids; akuammine, akuammicine, akuammidine and akuammiline as major compounds (10). In a study by De Campos *et al*. 2020 aqueous seed extract of *P. nitida* was shown to alleviate dyslipidaemia, hyperglycaemia, and pro-oxidant status associated with the intake of a high-fat high fructose diet (13). *P. nitida* leaf extract has been shown to ameliorate oxidative stress and modulates insulin signalling pathway in high fat-diet/STZ-induced diabetic rats (14). A study has also shown that

Picralima nitida seed and pod have hepatoprotective activity at 400 mg/kg once daily for 14 days in CCl4 induced liver damage or injury in animal model (15).

Figure 1. Picture of *Picralima nitida* leaves.

This study aims to undertake the pharmacognostic analysis and evaluate the methanol extract and fractions of the leaf on the potential hepatoprotective effect against carbon tetrachloride (CCl_4) induced liver damage in Swiss albino mice.

Materials and Methods

Chemicals

Chemicals and experimental reagents used include methanol, n-Hexane, butanol, ethyl acetate, diethyl ether (JHD, China), Tween-80, Ascorbic acid, Fehling's solution (A&B), Ammonia solution, Millions reagent, ferric chloride (Griffin & George, England), thiobarbituric acid (TBA) (Guangdong Guanghua Chemical Factory Co., Ltd, China), HCL, Alkaline phosphatase reagent kit (Teco Diagnostics, USA), Aspartate aminotransferase reagent kit (Randox Laboratories limited, United Kingdom), Alanine aminotransferase reagent kit (ALT, Randox Laboratories Limited, United Kingdom), etc. All solvents/reagents purchased were of analytical grade. All laboratory reagents were freshly prepared and freshly distilled water was used when required.

Animals

Swiss albino mice (25–30 g) were employed for the study. All the animals were obtained from the Animal House of the Department of Pharmacology and Toxicology, Enugu State University of Science and Technology, Enugu State. The animals were housed in standard laboratory conditions. The animals were allowed free access to food and water and all animal experiments were conducted in compliance with the NIH guide for the care and use of laboratory animals (National Institute of Health (NIH) (2011) Pub No: 85-23). Institutional animal ethics approval was obtained (ESUT/AEC/0138/AP096).

Collection of Plant Material

The leaves of *P. nitida* were purchased in July 2021 from Ibadan in Oyo State, Nigeria. The plant was identified and authenticated by a taxonomist Mr Felix Nwafor at the department of Pharmacognosy and Environmental Science, University of Nigeria, Nsukka, and the herbarium specimen was deposited at the University of Nigeria, Nsukka, Enugu State, Nigeria, with voucher number PCG/UNN/0442.

Preparation and Extraction of Plant Material

The leaves of *P. nitida* collected were cleaned to remove contaminants and air dried under room temperature. They were further pulverized to a fine powder using a mechanical grinding machine. The powdered leaves were stored in an air-tight container till further use. A 1.5 kg amount of the powder was extracted in 4.5 L of 99% methanol by cold maceration for 72 hours with intermittent shaking. The solutions were filtered with Whatman filter paper and the filtrates obtained were concentrated using rotary evaporator at 40 °C.

Fractionation of Plant Extract

The crude methanol extract of *P. nitida* (107.68 g) was subjected to liquid-liquid partition successively with n-hexane, butanol, ethyl acetate and water in increasing order of polarity to obtain n-hexane, butanol and ethyl acetate and water fractions respectively. The fractions were concentrated using rotary evaporator at 40 °C.

Microscopy Evaluation

The qualitative and quantitative microscopy was done according to the method described by Nwafor *et al*. (2019). The Freehand section of the leaves was prepared by clearing method and stained with safranin solution to reveal the epidermal cells, stomata type and size, stomata density and index, trichome parameters and vein islet numbers. They were viewed under a light phase contrast microscope (Motic B3, Motic Carlsbad, CA, USA) at x 40, x 100, and x 400 magnifications and photomicrographs were taken with a Moticam 2.0 image system with software (Motic Carlsbad, CA, USA). All parameters were observed on both the adaxial and abaxial surfaces of the leaves. A chemomicroscopy examination was also conducted on the leaf powder to determine the presence of starch, calcium oxalate crystals, and lignified vessels using standard methods.

Physicochemical Studies

The physicochemical analysis of the leaf powder was carried out to determine the total ash, acid-insoluble ash, water-soluble ash and extractive value using standard methods (16).

Acute Toxicity

Acute toxicity tests were performed in mice according to the method described by Lorke (17).

Table 1. The Yields of Methanol Extract and Fractions of *P. nitida* leaf.

Extracts/Fractions	Yield (g)	Yield (%w/w)
Methanol extract	107.68	8.61a
N-hexane fraction	25.99	29.68b
Butanol fraction	14.84	16.94b
Ethyl acetate fraction	38.58	44.05b
Water fraction	8.17	9.33b

Note: [a]Yield calculated from 1250 g of powdered leaves and [b]Yield calculated from 87.58 g of methanol extract.

Experimental Design

Sixty Swiss albino mice were divided into seven groups. Groups one to five have ten mice each, while groups six and seven have 5 mice each. Five of the mice in group one were pre-treated with 200 mg/kg of the methanol extract, while five were pre-treated with 400 mg/kg of the methanol extract. Five of the mice in group two were pre-treated with 200 mg/kg of the n-hexane fraction, while five were pre-treated with 400 mg/kg of the n-hexane fraction. Five of the mice in group three were pre-treated with 200 mg/kg of the ethyl acetate fraction, while five were pre-treated with 400 mg/kg of the ethyl acetate fraction. Five of the mice in group four were pre-treated with 200 mg/kg of the butanol extract, while five were pre-treated with 400 mg/kg of the butanol fraction. Five mice in group five were pre-treated with 200 mg/kg of the water fraction, while five were pre-treated with 400 mg/kg. Group six was pre-treated with 100 mg/kg of ascorbic acid (positive control), while group seven served as the negative control (2 % tween 80 and 80 ml/kg of water). After 14 days, the animals in all the groups except those in the negative control group were administered CCl_4 (49 ml dissolved in 1 ml of olive oil) through intraperitoneal injection.

Blood samples were collected from all the animals through the orbital sinus after 24 hours. They were centrifuged (model 7GL-20M, China) at 3000 rpm for 10 minutes, and the supernatant was decanted to get the serum. The serum was used to estimate the serum liver marker enzymes, which are Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), Alanine transaminase (ALT), and lipid peroxidation (MDA)

Statistical Analysis

The results were analysed using SPSS version 16 and presented as mean ± standard error of mean (SEM). Significance between control and extract-treated groups were determined using one-way analysis of variance (ANOVA). Differences between means were considered statistically significant at $p < 0.05$.

Results

Yield of *P. nitida* Methanol Extract and Fractions

The yield in gram and percentage of the methanolic leaves extract and fractions of *P. nitida* are presented in **Table 1**.

Fresh leaf microscopic analysis of *P. nitida*

The result of the fresh leaf microscopic examination of the fresh leaf of *P. nitida* is present in **Figures 2** and **3**. The transverse section of the leaf is presented in **Figure 4**, showing the upper epidermis, palisade tissue, collenchyma, xylem, lower epidermis and phloem. Presented in **Figure 5** is the Chemomicroscopy of the powder showing reticulate type of vessel elements aligned with fibre and parenchyma cells. The vessel and fibre elements are lignified while the ray parenchyma is not lignified. Presented in **Figure 6** is the Photomicrograph of the powdered leaf of *P. nitida* showing a pack of palisade tissue, prism-shaped calcium oxalate crystal and isolated and coiled fibre elements. Presented in **Table 2** and **3** are the results of the quantitative and qualitative microscopy respectively.

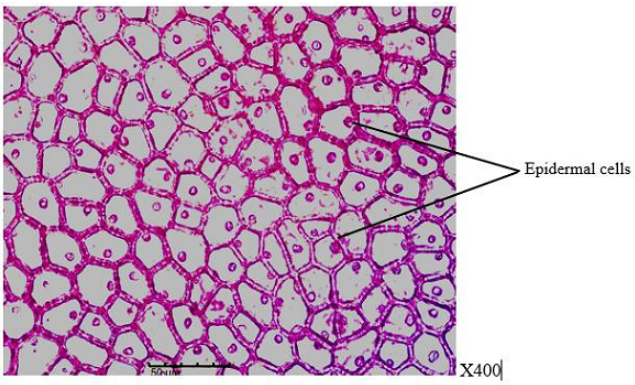

Figure 2. Adaxial (upper) surface of the leaf of *P. nitida* showing polygonally-shaped epidermal cells.

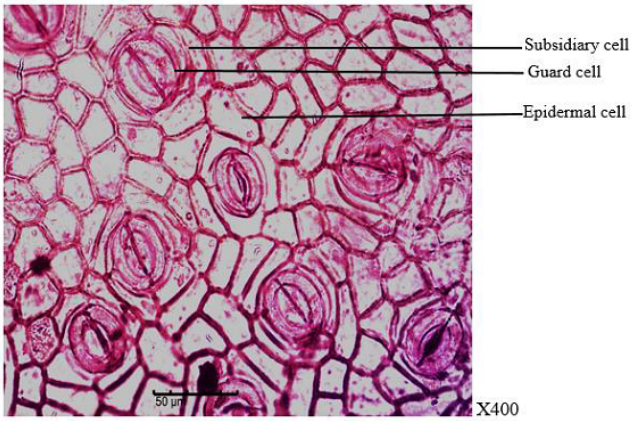

Figure 3. Abaxial (lower) surface of the leaf of *P. nitida* showing polygonally-shaped epidermal cells. Stomata are present (paracytic type). Trichomes are absent.

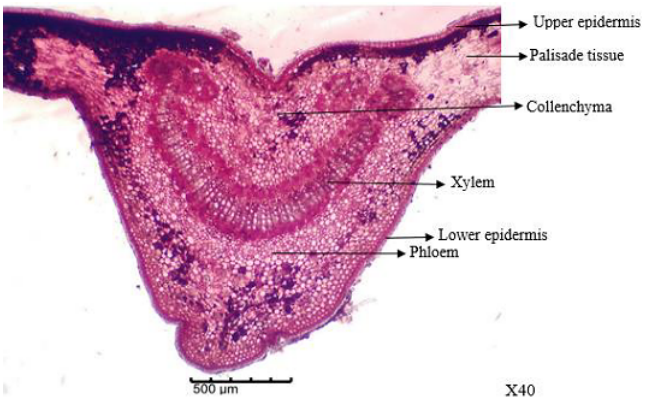

Figure 4. Transverse section of midrib of leaf of *P. nitida*.

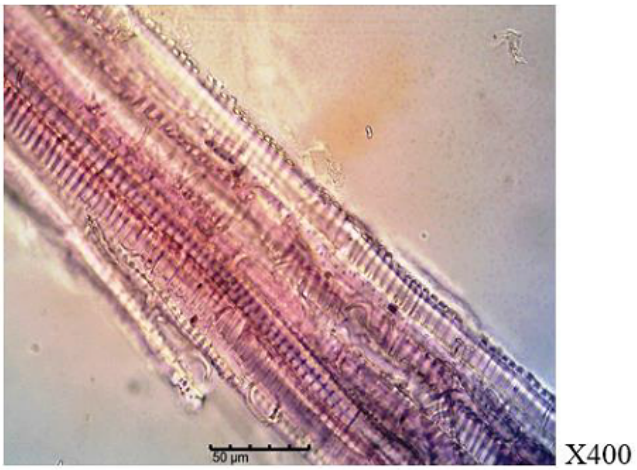

Figure 5. Chemomicroscopy of powdered leaf of *P. nitida*.

Figure 6. Photomicrograph of the powdered leaf of *P. nitida*.

Table 2. Quantitative microscopic result.

Parameters	Range
Stomata density	45.10 ± 1.96 mm-2
Stomata length	39.17 ± 2.16 µm
Stomata width	29.81 ± 1.52 µm
Stomata size	1165.04 ± 69.95 µm2
Vein islet number	6.24 ± 0.13 mm-2
Veinlet termination number	8.84 ± 1.33 mm-2
Palisade ratio	11.25 ± 0.25

Table 3. Result of powder chemomicroscopy of the leaf of *P. nitida*.

Parameter	Reagent(s)	Result
Starch grains	Iodine solution	Present
Lignified tissues	Conc. HCl + Phloroglucinol	Present
Calcium oxalates	Iodine solution Conc. Sulphuric acid	Present; Prism shape
Tannin	Ferric chloride	Present
Cellulose	Zinc chloride; Conc. Sulphuric acid	Present
Gum/Mucilage	Ruthenium red	Absent
Protein	Biuret reagent; Nihydrin	Present
Oil	Sudan III reagent	Present

Physicochemical Studies of the Powdered leaves of *P. nitida*

The result of the physicochemical analysis of the powder is presented in **Table 4**, showing the physicochemical parameter values of *P. nitida* leaf powder.

Table 4. Physicochemical analysis result.

Parameters	Result (%w/w)
Moisture content	11.75 ± 0.75
Total ash	8.50 ± 0.01
Acid insoluble ash	9.50 ± 0.00
Water soluble ash	4.00 ± 0.02
Alcohol Extractive value	13.75 ± 0.01
Water Extractive value	11.00 ± 0.01

Acute Toxicity of Methanol Extract of *P. nitida* Leaf

The result of the acute toxicity study of the methanolic extract of the leaf of *P. nitida* show that at 2000, 3000, 4000 and 5000 mg/kg body weight mice, no sign of toxicity was observed and no death was recorded.

Table 5. Effects of the methanol extract and fractions of *P. nitida* leaves on Alanine transaminase level in CCl_4 induced hepatotoxicity in mice.

Sample	Alanine transaminase (ALT) level (U/L)		Control sample	ALT level (U/L)
	200 mg/kg	400 mg/kg		
Methanol extract	76.04 ±11.76	46.18 ±7.33	Ascorbic acid	31.36 ± 3.77
Hexane fraction	130.82 ± 4.71	129.86 ±1.45	Negative	128.50 ± 12.85
Butanol fraction	80.46 ± 6.45	77.54± 4.74	Naïve	28.04 ± 1.75
Ethyl fraction	33.64 ± 0.70	32.07± 2.80		
Water fraction	116.18 ± 6.45	113.25± 4.74		

Table 6. Effects of the methanol extract and fractions of *P. nitida* leaves on Aspartate aminotransferase level in CCl_4 induced hepatotoxicity in mice.

Sample	Aspartate aminotransferase (AST) level (U/L)		Control sample	AST level (U/L)
	200 mg/kg	400 mg/kg		
Methanol extract	150.00 ± 7.40	118.95 ±8.54	Ascorbic acid	63.26 ± 2.05
Hexane fraction	307.16 ± 12.17	292.84 ±15.40	Negative	281.89 ±16.13
Butanol fraction	219.26 ± 11.13	199.37 ± 6.02	Naïve	55.47 ± 3.13
Ethyl fraction	99.79 ± 2.37	69.89 ± 6.35		
Water fraction	241.79 ± 6.95	214.42 ± 5.44		

Table 7. Effects of the methanol extract and fractions of *P. nitida* leaves on Alkaline Phosphatase level in CCl_4 induced hepatotoxicity in mice.

Sample	Alkaline Phosphatase (ALP) level (U/L)		Control sample	ALP level (U/L)
	200 mg/kg	400 mg/kg		
Methanol extract	104.20 ± 12.33	100.25 ± 13.05	Ascorbic acid	76.67 ± 5.99
Hexane fraction	135.31 ± 11.04	134.94 ±13.14	Negative	127.65 ± 22.84
Butanol fraction	101.48 ± 19.78	97.90 ± 9.98	Naïve	54.57 ± 20.12
Ethyl fraction	75.31 ± 3.70	72.84 ± 8.42		
Water fraction	120.25 ± 22.72	91.11 ± 19.21		

Table 8. Effects of the methanol extract and fractions of *P. nitida* leaves on Malondialdehyde level in CCl_4 induced hepatotoxicity in mice.

Sample	Malondialdehyde (MDA) level (µm/ml)		Control sample	ALP level (µm/ml)
	200 mg/kg	400 mg/kg		
Methanol extract	0.83 ± 0.03	0.81 ± 0.03	Ascorbic acid	0.70 ± 0.03
Hexane fraction	1.18 ± 0.04	1.21 ± 0.04	Negative	1.16 ± 0.11
Butanol fraction	0.89 ± 0.06	0.87 ± 0.04	Naïve	0.53 ± 0.04
Ethyl fraction	0.81 ± 0.03	0.71 ± 0.05		
Water fraction	1.01 ± 0.06	0.97 ± 0.02		

Hepatoprotective Activity

The estimation of enzymes in the serum is a useful quantitative marker of the extent and type of hepatocellular damage. The mice administered with CCl_4 caused significant liver damage and necrosis of cells as evidenced by the elevated serum hepatic enzymes (ALT, AST, ALP and MDA) as shown in the negative groups in **Figures 7** - **10**. The level of enzyme markers ALT, AST, ALP and MDA in normal (Naïve) mice were found to be 28.04±1.75,

55.47±3.13, 54.57±20.12 U/L and 0.53±0.04 µm/ml respectively; as expected, CCl_4 caused their elevation to 128.50±12.85, 281.89±16.13, 127.65 ± 22.84 U/L and 1.16±0.11 µm/ml respectively, as seen in the negative group. Pre-treatment with leaf extract and fractions significantly ($p < 0.05$) reduced their elevations for the methanolic extract and ethyl acetate fraction treated groups (see **Table 5** - **8**).

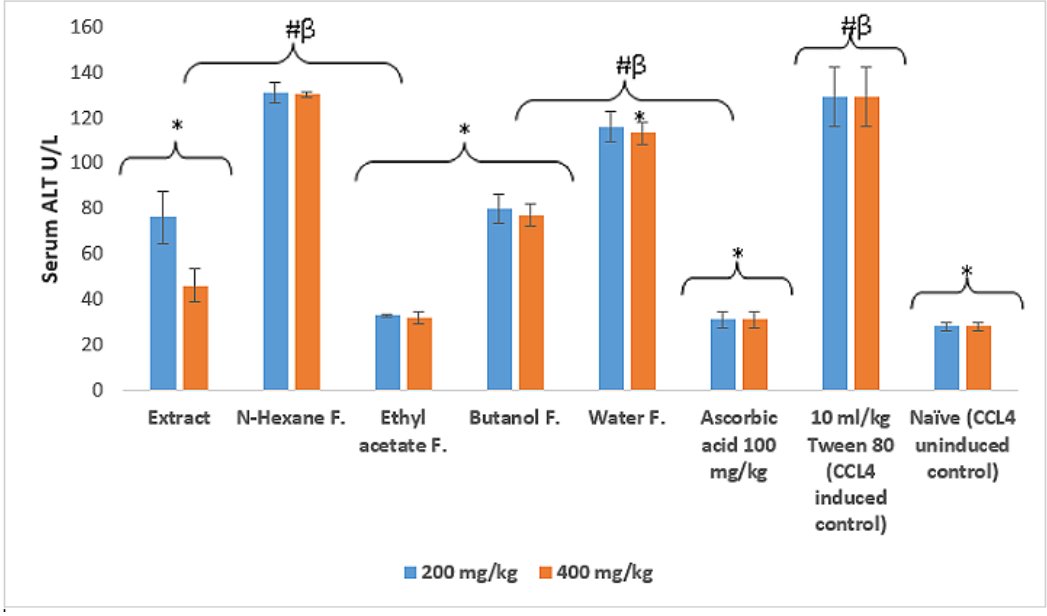

Figure 7. Effect of extract and fraction on serum Alanine aminotransferase (ALT). **Note:** *$p < 0.05$ compared to 10 ml/kg tween 80 CCL_4 induced vehicle control group; $^{\#}p < 0.05$ compared to 100 mg/kg ascorbic acid; $^{\beta}p < 0.05$ compared to naïve CCl_4 uninduced control group.

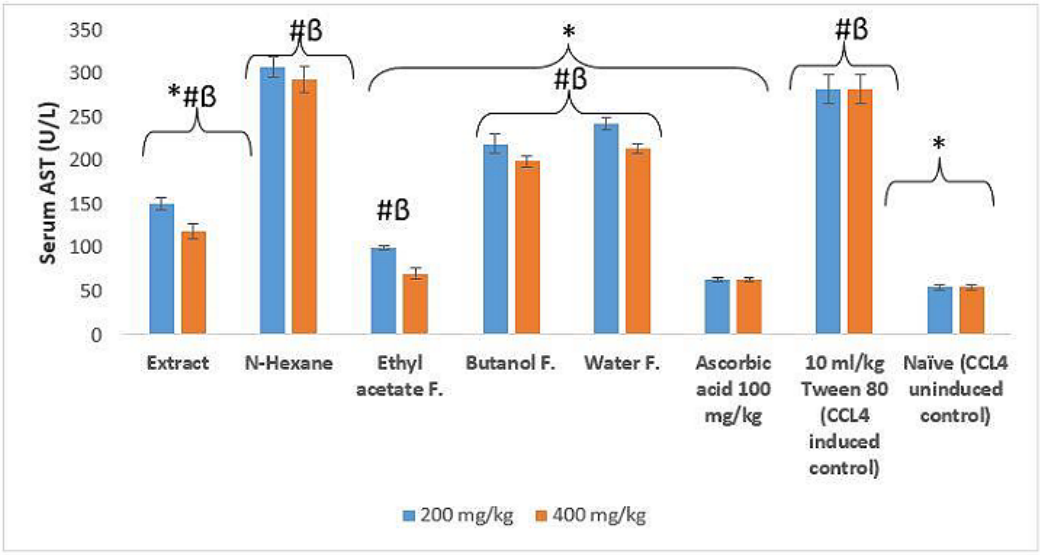

Figure 8. Effect of extract and fraction on serum Aspartate aminotransferase (AST). **Note:** *$p < 0.05$ compared to 10 ml/kg tween 80 CCL_4 induced vehicle control group; #$p < 0.05$ compared to 100 mg/kg ascorbic acid; $^{\beta}p < 0.05$ compared to naïve CCl_4 uninduced control group.

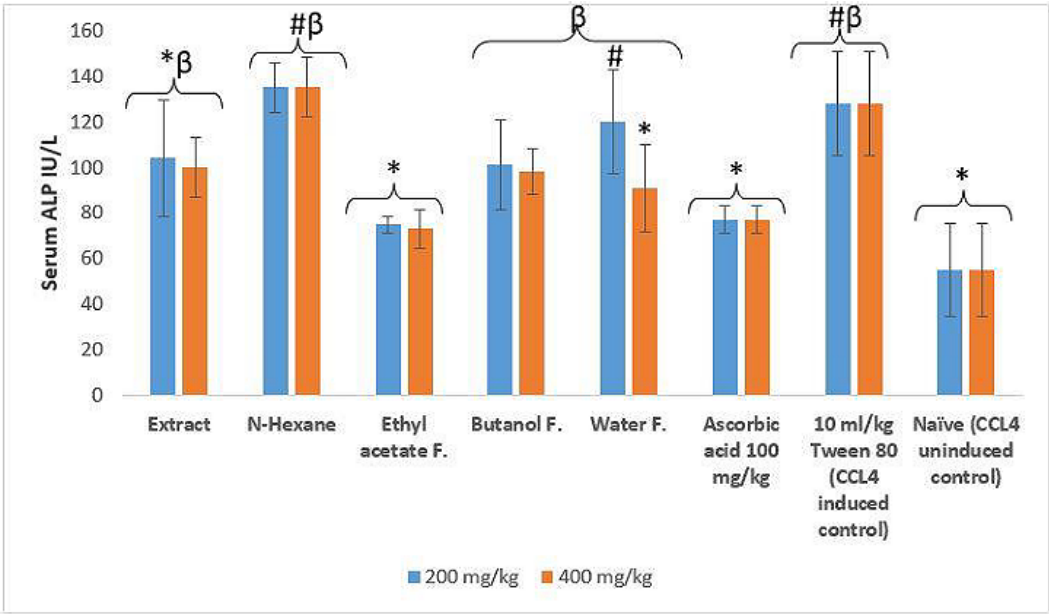

Figure 9. Effect of extract and fraction on serum Alkaline Phosphatase (ALP). **Note:** *$p < 0.05$ compared to 10 ml/kg tween 80 CCL_4 induced vehicle control group; #$p < 0.05$ compared to 100 mg/kg ascorbic acid; $^{\beta}p < 0.05$ compared to naïve CCl_4 uninduced control group.

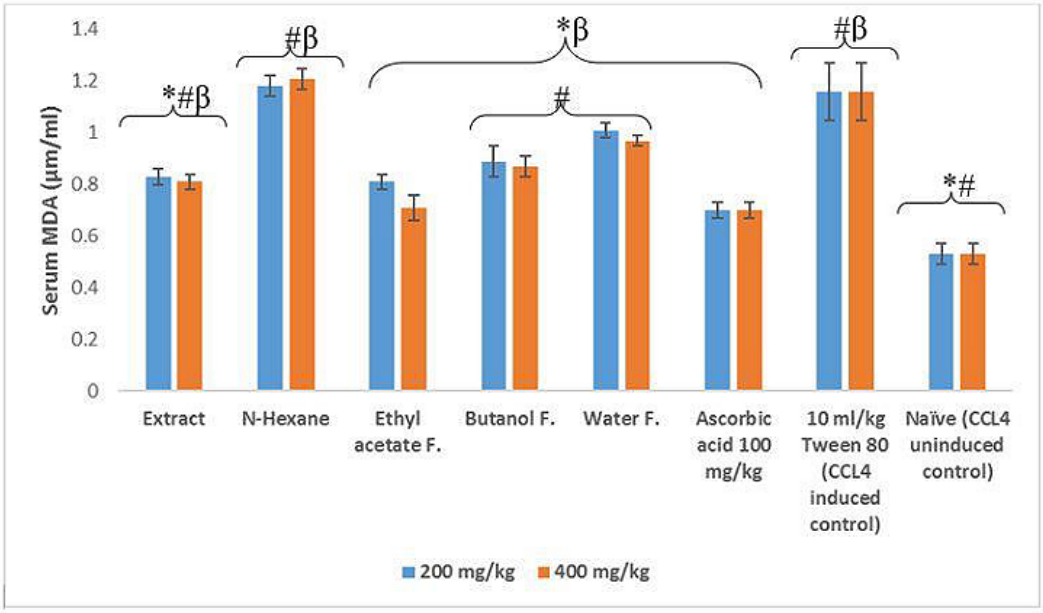

Figure 10. Effect of extract and fraction on Malondialdehyde (MDA). **Note:** *$p < 0.05$ compared to 10 ml/kg tween 80 CCL_4 induced vehicle control group; #$p < 0.05$ compared to 100 mg/kg ascorbic acid; $^{\beta}p < 0.05$ compared to naïve CCl_4 uninduced control group.

Discussion

This paper reports the methanolic extractive value and different fractions yield of *P. nitida* Leaves; the pharmacognostic parameters of the fresh and powdered leaves of *P. nitida*, comprising of microscopy, chemomicroscopy and physicochemical studies; acute toxicity studies and Hepatoprotective effect of the methanolic extract and fractions of *P. nitida*.

The result presented in **Table 1** shows the percentage extractive value of different solvents used for extraction. The extraction efficiency of solvents varies depending on the nature of the compound being extracted, solubility, and polarity of the solvent used. In this case, different solvents were used, and the results show that ethyl acetate has the highest extractive value (44.05 %), followed by n-hexane (29.68 %), butanol (16.94 %), methanol (8.6 %), and

water (9.33 %). Ethyl acetate is a common solvent used for the extraction of natural products due to its low toxicity, low boiling point, and high solvating power for non-polar and polar compounds (18). This solvent has been used to extract various bioactive compounds from different plant materials, including flavonoids, phenolics, and alkaloids (18). N-hexane is a non-polar solvent that is widely used for the extraction of lipids, fatty acids, and essential oils from plant materials (19). This solvent has a high solubility for non-polar compounds and is often used in combination with polar solvents to obtain a broader range of compounds from plant materials (19). Butanol is a polar solvent that can dissolve both polar and non-polar compounds. It is commonly used for the extraction of flavonoids, phenolics, and other bioactive compounds from plant materials. Methanol is a polar solvent that is commonly used for the extraction of polar compounds such as alkaloids, tannins, and flavonoids (19). However, methanol is toxic and can have adverse effects on human health, making it less desirable as a solvent for extraction. Water is a polar solvent that is commonly used for the extraction of polar compounds such as sugars, amino acids, and organic acids (19). However, water has limited solubility for non-polar compounds and is often used in combination with other solvents to obtain a broader range of compounds from plant materials. The results presented in **Table 1**, suggest that different solvents have varying extraction efficiencies depending on the nature of the compound being extracted. Ethyl acetate, n-hexane, and butanol have high extraction efficiencies for different types of compounds, while methanol and water have lower extraction efficiencies for non-polar compounds.

Microscopic evaluation is important in determining the identity and purity of a plant material. They are used as diagnostic features for microscopic evaluation of plants (20). The fresh leaf microscopic feature revealed a polygonal-shaped epidermal cell (abaxial) with straight anti-clinical wall and lignified cell wall (adaxial). Stomata and trichomes are absent on the adaxial surface while stomata are present on the abaxial. Transverse section of the leaf revealed the presence of a single layered epidermises. A sickle shaped vascular bundle is present showing lignified xylem tissue and a non-lignified ground tissue and pith. This result agrees with the report of Bruce *et al*. (2022) (21). Chemomicroscopy of the leaf revealed the presence of starch grains, lignin, cellulose, calcium oxalate (prism shaped), tannins, proteins and oils.

The physicochemical results reported for *P. nitida* as shown in **Table 5** provide information about its chemical and physical properties. Physicochemical analysis is used to determine the purity and quality of a drug (22). The proximate composition of *P. nitida* leaf extract contains moisture content 11.75%, total ash 8.5%, acid insoluble ash 9.5%, water soluble ash 4.0%, alcohol soluble extractive value 13.75% and water soluble extractive value 11.0%. The result is in contrast when compared with the proximate composition of Bruce *et al*. (2022) (21). High moisture content means that the drug cannot be stored for a longer period which could enhance the breakdown of crucial bioactive compound (21). High ash value is due to contamination and presence of impurities (23). Extractive value helps to evaluate chemical constituents of a drug (21) and helps in estimating specific constituents that are soluble in a particular solvent (24).

CCl_4 is a well-known compound that is often utilized to induce liver damage in experimental animal models for studying the development of hepatic steatosis caused by xenobiotics (25, 26). This liver injury is attributed to the oxidative stress induced by reactive oxygen species (ROS) that can produce harmful lipid intermediates. In a study conducted by Weber and colleagues (27), they found that the consumption of CCl_4 leads to the activation of the cytochrome system (specifically CYP2E1), which generates trichloromethyl radicals (CCl_3+). The formation of these reactive intermediates through reductive metabolism results in toxicity and can cause the leakage of serum enzymes, lipid peroxidation, depletion of antioxidant capacity, and hepatic necrosis around the central vein (28).

Over the course of approximately thirty years, researchers have discovered that extracts from various natural sources possess hepatoprotective properties, which can mitigate CCl_4-induced toxicity at varying doses. This is achieved through the reduction of oxidative stress on liver enzymes (29). The primary mechanism through which herbal plants offer protection against CCl_4-induced hepatotoxicity is by inhibiting the activity of microsomal enzymes using their phytochemical components (30, 31). These phytochemicals have the ability to curb the formation of free radicals and halt lipid peroxidation through their antioxidant properties (32). Additionally, they promote the regeneration of liver cells and exhibit radical scavenging properties, while also enhancing the anti-inflammatory capacity of liver cells in response to CCl_4-induced inflammation (33).

The results obtained from the administrations of methanol extract, n-hexane fraction, Butanol fraction and Ethyl acetate fraction of *P. nitida* which is presented in **Figures 6**, **7**, **8** and **9**. The administration of CCl_4 produced a hepatotoxic effect which is evident by a significant increase in serum liver function enzymes. However, the administration of the extract and the fractions were able to lower the increase in the liver function enzymes. This is an indicator of their hepatoprotective activity.

The extract and fractions with the exception of n-

hexane, produced a significant reduction in serum ALT compared with the vehicle control group. The fractions in their increasing order gave a better hepatoprotective activity; ethyl acetate fraction > butanol fraction > water fraction. The extract gave a significant increase when compared with the positive control (standard) and uninduced naïve group. The fractions with the exception of ethyl acetate produced a significant increase compared with the positive and naïve control. Ethyl acetate gave no significant difference which means that ethyl acetate fraction restores increase serum ALT to normality like the standard and an unaffected individual.

The extract and fractions with the exception of n-hexane fraction produced a significant reduction in the increased serum AST compared with the vehicle induced control in the order; ethyl acetate fraction> extract>butanol fraction> water fraction. The extract gave a significant reduction in the increased serum AST and also, produced a significant increase compared with the positive and naïve control. However, ethyl acetate fraction at 400 mg/kg produced no significant difference compared with the standard (ascorbic acid) and naïve (normal).

The extract with the butanol and water fraction, produced a slight reduction in the increased serum ALP compared with the vehicle control. While ethyl acetate fraction produced a significant reduction, n-hexane fraction gave a significant increase compared with the vehicle control. The extract and fractions produced a significant increase when compared to the positive control (ascorbic acid) with the exception of ethyl acetate fraction which produced no significant difference. Ethyl acetate fraction when compared to the naïve uninduced control, produced a significant increase. This means it could not restore to normality the serum ALP.

CCl_4 was able to produce a significant lipid peroxidation as seen by the significant difference between the two controls (vehicle CCl_4 induced and naïve uninduced control). There is a significant effect in the extract and ethyl acetate fraction but n-hexane produced no significant effect. There was a significant reduction when butanol and water fraction was compared to the vehicle control. Ethyl acetate at 400 mg/kg, however, produced no significant difference compared with the positive control unlike the extract and other fractions which produced a significant increase. However, none of the fractions nor extract could restore lipid peroxidation when compared with the uninduced control.

Conclusion

The current research offers scientific proof supporting the use of *P. nitida* leaves as a viable alternative in pharmacological treatment for liver disorders. Isolation of the bioactive constituents of the ethyl acetate fraction of the methanol extract of *P. nitida* will be carried out, and the isolated phytocompounds evaluated, to identify the constituent(s) responsible for the hepatoprotective effect.

Declarations

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Contribution: Conceptualization, Formal analysis, Project administration, Resources, Supervision, Validation.

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Conflict of Interest

The authors declare no conflicting interest.

Data Availability

The unpublished data is available upon request to the corresponding author.

Ethics Statement

The study was approved by the Institutional Animal Ethical Committee with approval letter number of ESUT/AEC/0138/AP096.

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